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MICROBIOLOGY

Study Adds Carbs To Immune Cell Menu

Findings About Way Carbohydrate Antigens Power Up T Cells Raise Hope for New Vaccines

Invading pathogens may be consumed whole by phagocytes or skewered by a marauding band of antimicrobial proteins, the complement system. Others are chewed up into tiny bits by immune system scouts and spit out in the form of antigens that trigger T cells to hunt down similar pathogens. For years, the antigen-presenting scouts have been thought to dine exclusively on proteins, avoiding the gelatinous carbohydrate coat that surrounds bacteria. So entrenched is this idea—and its corollary, that only proteins can activate T cells—that most immunologists have not questioned it.

It now appears that antigen-presenting cells consume a more eclectic diet than previously thought. Brian Cobb, Dennis Kasper, and their colleagues have essentially caught the cells in the act of taking in and degrading a set of carbohydrates. What is more, the carbohydrate antigens appear to follow essentially the same processing route as proteins, the well known MHC II pathway. And they are spewed out onto the surface of antigen-producing cells, where they activate T cells, just like protein antigens.

The findings, reported in the May 28 *Cell*, could open the door to new methods for rousing the immune system to fight disease through vaccines and antimicrobial therapies. "What this does is broaden the scope of molecules that the host can actually recognize," said Cobb, HMS research fellow in microbiology and

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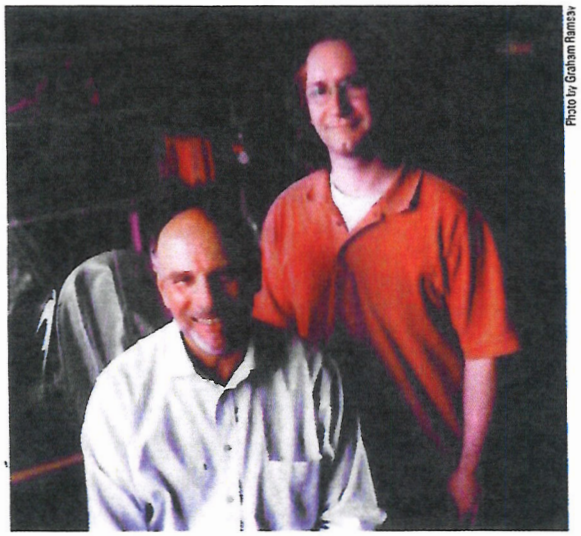

Photo by Graham Ramsay

"Vaccine target research tends to focus on proteins—people tend to ignore the other molecules. Carbohydrates solidly fall into that category," said Brian Cobb (right) with Dennis Kasper. "I think that our findings open up a whole new world, not just of polysaccharide antigens, but other classes of molecules that might be involved."

Carbs on the Menu

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molecular genetics.

Chinks in the protein-only dogma began to appear about a decade ago when Kasper, the William Ellery Channing professor of medicine at HMS, and his colleagues stumbled upon a class of bacterial carbohydrates—carrying a peculiar constellation of electrical charges—that appeared to activate T cells. Most carbohydrates consist of chains of repeating sugars that carry a negative charge or no ionic charge at all. Each of the sugars in the T cell–activating variant carried alternating positive and negative charges.

So far it appears that only these alternatively charged, or zwitterionic, carbohydrates are processed by antigen-presenting cells. But Cobb, Kasper, and their colleagues believe this characteristic might be imparted for therapeutic purposes. "For example, it might be possible to take other polysaccharides and maybe add or modify parts to give a zwitterionic character to part of the molecule," said Kasper, who is also an HMS professor of microbiology and molecular genetics.

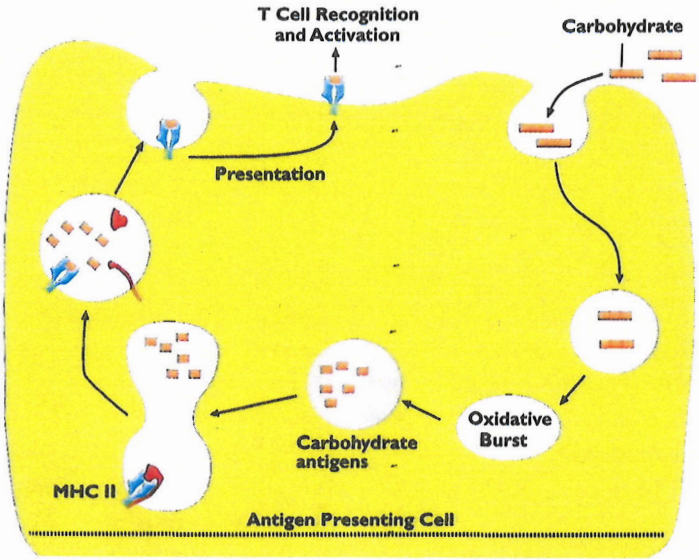

Image courtesy of Brian Cobb

Antigen-presenting cells process carbohydrates and proteins using nearly the same MHC II pathway. Soon after entering the cell, carbohydrates are broken down into antigen-size bits by the oxidative burst. (Proteins are degraded by proteases.) Antigens are then loaded onto MHC II molecules and presented on the surface of the cell, where they activate nearby T cells.

Building a Better Vaccine

Finding microbial molecules that provoke a strong immune response while doing no damage is the dream of vaccine research. The carbohydrates making up bacteria's gel-like coat have long been considered promising candidates. They mimic polysaccharides found on host cells, enabling the microbes to slip into the body unnoticed by phagocytes and the complement system, and on their own, they do no harm. Though some do provoke immune cells to make pathogen-fighting antibodies, the response is relatively weak. To make a stronger vaccine, T cells need to stimulate antibody-producing B cells. Vaccine designers have tried tying carbohydrates to T cell–activating protein antigens, with some success. The haemophilus influenza vaccine is an example of such a conjugated vaccine. But there are a limited number of peptides that can be used to make them.

"It would be neat to be able to take a polysaccharide, modify it, and not have to do the conjugation. You would get T cell help without doing the work. But this is total speculation," said Kasper.

When Cobb arrived in Kasper's lab in 2001, hints had already emerged that the zwitterionic carbohydrates might be activating T cells through antigen-presenting cells. Working with Kasper; Qun Wang, research fellow in microbiology and molecular genetics; and Arthur Tzianabos, HMS associate professor of medicine, Cobb first tested whether the sugar molecules actually do enter antigen-presenting cells, and if so, whether they are cut down to antigen size.

Fingering a Trigger

Using a combination of biochemistry and confocal microscopy, the researchers confirmed the presence of appropriately sized carbohydrate fragments inside and also on the surface of antigen-presenting cells. Using special markers, they tracked the fragments' passage through the various vesicular com-

partments of the MHC II pathway. But they found an intriguing twist.

Normally, after entering antigen-presenting cells, bacteria are killed by an oxidative burst, and their proteins are whittled down to antigen size by a set of proteases. The peptide antigens are then mounted on MHC II molecules and shuttled through a series of vesicles to the surface of the cell, where they are displayed to passing T cells. Cobb looked for an enzyme that would cut the polysaccharide chains into smaller bits. "I was never able to find anything that would do it," he said.

Thinking that the oxidative burst might play a role, the researchers hit the polysaccharides with the oxidative agent ozone. It did the trick—the sugars were cut down to antigenic size. To confirm oxidation's role in vivo, they exposed mice lacking a key oxidative compound, nitric oxide, to the zwitterionic carbohydrates. The knockout mice's T cells remained inactive.

Additional experiments revealed the zwitterionic sugars binding to MHC II molecules in normal cultured cells, but not in the cells of the nitric oxide–deprived mice. As for how the binding occurs, there are curious clues. Most peptide antigens fit into MHC II molecules like a hotdog in a bun. According to the researchers, the sugar antigens may not be mounted in this fashion. "It is probably charge based, at least partially, but we do not know much more than that," said Cobb. "The more we know, the more we can apply it to taking another polysaccharide and making sure it has the same character."

—Misia Landau